

Inorganic Mercury and Methylmercury in Placentas of Swedish Women

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We determined levels of inorganic mercury (I-Hg) and methylmercury in placentas from 119 Swedish women, not selected with respect to high exposure of mercury. Our objective was to relate placental Hg species with maternal and fetal blood concentrations and to evaluate possible associations with selenium. We performed the analyses using automated alkaline solubilization/reduction and cold-vapor atomic fluorescence spectrophotometry. I-Hg levels in placenta increased with an increasing number of maternal dental amalgam fillings ($p < 0.001$). Despite placental accumulation (median, 1.3 $\mu\text{g}/\text{kg}$; range, 0.18–6.7 $\mu\text{g}/\text{kg}$ wet weight), a substantial fraction of maternal blood I-Hg, probably as Hg^0 , reached the fetus. Although MeHg transferred easily to the fetus, it also accumulated in the placenta. On average, 60% of placental Hg was in the form of MeHg. The median concentration was 1.8 $\mu\text{g}/\text{kg}$ (range, 0–6.2 $\mu\text{g}/\text{kg}$ wet weight), more than twice the maternal blood concentration. We found significant associations between MeHg and selenium in both maternal and umbilical cord blood but not in the placenta. The associations were particularly obvious in freshwater fish consumers, probably reflecting that fish is a source of both MeHg and selenium. We found no correlations between I-Hg and selenium. This study increases the understanding of Hg, in its different forms, in human placenta and how they are related to maternal and fetal exposure. **Key words:** fetal exposure, human, inorganic mercury, methylmercury, placenta, pregnancy, selenium. *Environ Health Perspect* 110:523–526 (2002). [Online 3 April 2002] <http://ehpnet1.niehs.nih.gov/docs/2002/110p523-526ask/abstract.html>

The placenta plays a central role for the fetus by providing nutrients and oxygen, but also by acting as a barrier to prevent passage of toxic substances, such as mercury. Both organic forms of Hg, such as methylmercury, and mercury vapor (Hg^0) are well-known neurotoxic agents, especially during early development (1), which is the most susceptible period.

Fish, mainly freshwater and large marine predatory species, is the dominant source of human exposure to MeHg (2,3). On the basis of recent data showing developmental effects of MeHg in children on the Faroe Islands (4), the U.S. National Research Council found the U.S. Environmental Protection Agency reference dose of 0.1 μg MeHg/kg body weight per day scientifically justifiable (1). Thus, women of childbearing age should avoid eating most predatory fish, which often contain 0.3–1.0 mg MeHg/kg (5,6). The main source of exposure to inorganic mercury (I-Hg) is dental amalgam fillings, which release Hg^0 (7,8). The intake and uptake of Hg^{2+} is negligible (9). The concentration of I-Hg in the fetus has been shown to be influenced by the number of dental amalgam fillings in the mother (10–12). An experimental study shows that the fetal brain distribution and effects are similar after exposure to MeHg and Hg^0 (13).

It is generally believed that MeHg and Hg^0 easily cross the placental barrier whereas Hg^{2+} is trapped (1,3). Inhaled Hg^0 is oxidized to inorganic Hg^{2+} by catalase already within the blood (3), but some Hg^0 remains in the circulation long enough to pass the

placental barrier (10,13,14). However, the reported studies in which the different forms of Hg in human placenta have been speciated (15–20) do not show consistently that I-Hg is the main Hg form in placenta.

To clarify this further, our aim was to determine placental accumulation of the different forms of Hg. We also related the placental concentrations of different Hg species to the maternal exposure, cord blood concentrations, and possible associations with selenium, which has been shown to protect against Hg toxicity both in human (*in vitro*) and animal studies (2,21,22).

Subjects and Methods

The study group consisted of pregnant women recruited from antenatal care units in Stockholm, Sweden (12,23,24). We collected blood samples at gestational week (GW) 36, and umbilical cord blood and placentas at delivery. We found that the women who donated placentas ($n = 119$) were representative of those entering the study ($n = 254$) with respect to age, parity, number of amalgam fillings, freshwater fish consumption, and Hg and selenium concentrations in their blood at GW 36. A self-administered questionnaire asked questions regarding number of dental amalgam fillings (0, < 10, or > 10 fillings) and frequency of freshwater fish consumption before pregnancy. In the analyses, we compared MeHg levels in women eating freshwater fish once a month or more with levels in those eating freshwater fish less often or never. The study was carried out in accordance with

the Helsinki declaration and was approved by the Ethics Committee at Karolinska Institutet.

We stored placentas frozen in acid-washed containers until preparation and homogenization, as described elsewhere (24,25). We divided each placenta into six sections and obtained three subsamples from each section. Concentrations of elements varied substantially among the subsamples. In order to obtain homogeneous samples, we homogenized placental tissues in a metal-free food processor (Hugin; Robot-Coupe, Le Perreux, France). Before homogenization, we sectioned placental samples from the maternal surface through the chorionic plate. We cut away the decidua basalis and chorionic plate and sampled primarily placental/trophoblastic tissues. We cleaned the specimens of blood and blotted them on filter paper. We determined the wet weight concentrations of I-Hg and total mercury (T-Hg) in whole placenta homogenate by alkaline solubilization/reduction and cold-vapor atomic fluorescence spectrophotometry (Merlin, PSA 10.023; P.S. Analytical Ltd., Orpington, Kent, UK) as previously described for blood (12). We treated subsamples of 2.0 g with 5.0 mL L-cysteine (1%), 5.0 mL NaOH (45%), and 6.0 mL NaCl (1%). To complete the solubilization, we heated the mixture at approximately 80–85°C for 30 min. We analyzed duplicate samples from all solubilizes. By subtracting the concentration of I-Hg from that of T-Hg, we achieved the concentration of organic Hg. We anticipated that the major part of the organic Hg fraction would be in the form of MeHg.

The limit of detection ($3 \times \text{SD}$ of the mean reagent blanks) for I-Hg in placenta was 0.10 $\mu\text{g}/\text{kg}$ (range, 0.04–0.16 $\mu\text{g}/\text{kg}$) and for T-Hg was 0.10 $\mu\text{g}/\text{kg}$ (range, 0.06–0.13 $\mu\text{g}/\text{kg}$). None of the sample concentrations was below the limit of detection. No reference sample with certified Hg concentration is available for placenta. To ensure the method's

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accuracy, we analyzed a reference blood sample (Seronorm 404108; Nycomed Co., Oslo, Norway) in duplicate twice in each of the seven analytical runs. No systematic change over time could be detected. The obtained mean value for T-Hg was 7.9 µg/L (range, 7.4–8.3 µg/L), well within the recommended range of 6.7–8.4 µg/L. Recovery studies using subsamples from one placenta homogenate spiked with 0.1 ng I-Hg and 0.1 ng MeHg gave average recoveries of 96% for both I-Hg and T-Hg. The precision of the method, expressed as coefficient of variation, was, respectively, 3.4% and 3.8% for I-Hg and T-Hg for the Seronorm sample and 9.4% and 9.2% for the placenta homogenate. Determination and analytical accuracy of Hg in blood and selenium in serum and placenta were satisfactory and have been previously described (12,24).

The data were not normally distributed. We tested differences between groups for significance using nonparametric tests (Kruskal-Wallis and Mann-Whitney *U*). However, when requirements of normally distributed residuals were met, we used Pearson's correlation and multiple regression analysis. We conducted all statistical analyses with SPSS, version 9.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows the concentrations of I-Hg and MeHg in the placentas, maternal blood, and umbilical cord blood. I-Hg levels in placenta were four times higher than those in

maternal and umbilical cord blood and were highly associated with maternal blood concentrations ($r^2 = 0.66$; $p < 0.001$; Figure 1)—but not influenced by maternal age or parity. We also found an association between I-Hg levels in placenta and in umbilical cord blood ($r^2 = 0.30$; $p < 0.001$). As shown in Figure 2, placental I-Hg levels increased markedly with increasing number of amalgam fillings ($p < 0.001$). We did not find any correlation between MeHg and I-Hg levels in placenta, before or after controlling for amalgam fillings.

The MeHg level in placenta was more than twice that in maternal blood but only slightly higher than that in umbilical cord blood. Figure 1 shows the correlation between MeHg levels in placenta and maternal blood ($r^2 = 0.72$; $p < 0.001$). Also the umbilical cord blood concentrations of MeHg were correlated with placental concentrations ($r^2 = 0.74$; $p < 0.001$). Placental MeHg levels increased with maternal age and with freshwater fish intake (multiple linear regression adjusted $r^2 = 0.14$; $p \leq 0.003$) but was not influenced by parity.

We found significant positive correlations between concentrations (mol/L) of selenium and MeHg levels in maternal blood or umbilical cord blood but not in placenta (Table 2). Those correlations were more pronounced in women reporting consumption of freshwater fish. We found a tendency for higher selenium concentrations in maternal serum of women reporting eating freshwater fish than in those who did not (mean, 81 µg/L, $n = 8$, and 72 µg/L, $n = 84$, respectively;

$p = 0.06$). The corresponding concentrations were, respectively, in umbilical cord blood 56 µg/L and 51 µg/L ($p = 0.28$) and in placenta 201 µg/kg and 195 µg/kg ($p = 0.31$) (24). We found no significant correlations between I-Hg and selenium levels in the different tissues.

Discussion

The present study of Swedish nonoccupationally exposed women with low intake of freshwater fish shows large variations of I-Hg and MeHg concentrations in placenta (range up to 7 µg/kg). Both were highly associated with the concentrations in maternal blood.

As previously shown in experimental studies (14), I-Hg accumulated in the placenta. The median placental concentration was four times higher than that in maternal blood and increased considerably with increasing number of amalgam fillings. Thus, it seems likely that the I-Hg bound in placenta originated from Hg^0 released from amalgam fillings and oxidized to Hg^{2+} by catalase in the blood. MeHg is known to slowly demethylate to inorganic Hg^{2+} in some tissues (12,26); however, the low I-Hg levels also in placentas with high MeHg concentrations indicated negligible demethylation of MeHg in the placenta in the present study. Hg^{2+} may be bound to metallothionein, which is rich in cysteine, in the placenta. Placentas have high levels of metallothionein (27) but relations to Hg levels have not been studied. However, *in utero* exposure to Hg^0 induced fetal brain metallothionein production in rats (28). Although we know little about the toxic effects of Hg on placental functions, *in vitro* studies suggest that Hg^{2+} can affect the transfer of amino acids, placental oxygen consumption (29), enzyme activity (30), and hormonal secretion (31).

Despite the marked placental accumulation of I-Hg, the concentration of I-Hg in umbilical cord blood was similar to that in

Table 1. I-Hg and MeHg concentrations in placenta, maternal blood (GW 36), and umbilical cord blood.

	No.	Median	5th percentile	95th percentile	Maximum
I-Hg					
Placenta (µg/kg)	119	1.3	0.40	4.7	6.7
Maternal blood (µg/L)	112	0.32	0.03	1.2	1.9
Cord blood (µg/L)	98	0.34	0.09	0.79	1.1
MeHg					
Placenta (µg/kg)	119	1.8	0.56	3.8	6.2
Maternal blood (µg/L)	112	0.73	0.19	2.1	2.8
Cord blood (µg/L)	98	1.4	0.26	3.8	4.8

Data on maternal and cord blood concentration have been published previously (12).

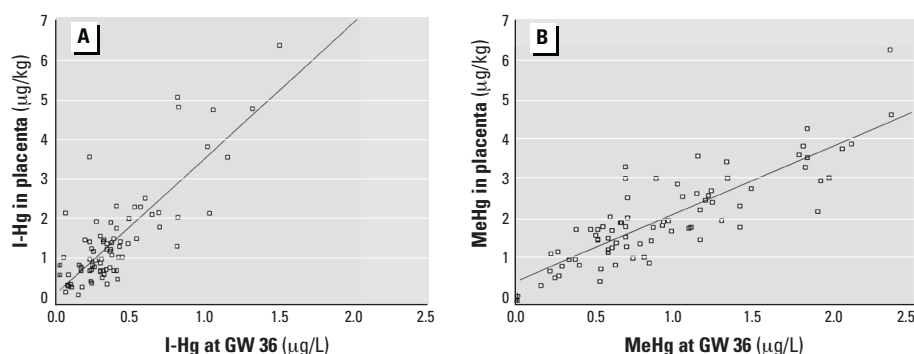


Figure 1. Regression between (A) placental and maternal I-Hg ($y = 0.32 + 3.3x$; $p < 0.001$) and (B) between placental and maternal MeHg ($y = 0.50 + 1.7x$; $p < 0.001$) at GW 36.

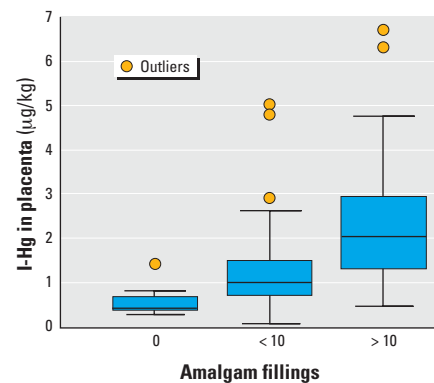


Figure 2. I-Hg in placenta in relation to maternal dental amalgam fillings: 0 fillings ($n = 8$), < 10 fillings ($n = 61$), and > 10 fillings ($n = 37$). Boxes depict 25th, 50th, and 75th percentiles, and whiskers depict minimum and maximum values, excluding outliers.

Table 2. Correlation coefficient (*r*) between blood MeHg and serum selenium in maternal blood, umbilical cord blood, and placenta.

	Maternal Se			Placenta Se			Cord Se		
	<i>r</i>	<i>p</i> -Value	No.	<i>r</i>	<i>p</i> -Value	No.	<i>r</i>	<i>p</i> -Value	No.
Maternal MeHg									
All	0.30*	0.008	78						
Freshwater fish	0.79*	0.034	7						
No freshwater fish	0.17	0.17	66						
Placenta MeHg									
All				0.19	0.057	106			
Freshwater fish				−0.21	0.56	10			
No freshwater fish				0.20	0.060	90			
Cord MeHg									
All							0.28*	0.014	77
Freshwater fish							0.43	0.39	6
No freshwater fish							0.20	0.10	66

**p* < 0.05.

maternal blood. We did not determine the forms of I-Hg passing to the fetus because the analytical method did not distinguish between Hg⁰ and Hg²⁺. However, because Hg²⁺ seems to cross the placental barrier only at high concentrations (14,29), most I-Hg likely passed to the fetus in the form of Hg⁰.

MeHg has been proposed to be readily transported across the placenta bound to thiol-groups in cysteine, thereby mimicking methionine (32). Indeed, we found higher MeHg concentrations in umbilical cord blood than in maternal blood. However, MeHg concentrations in placenta were about twice those in maternal blood, showing retention in placental tissue. In contrast to the hypothesis that I-Hg is the primary form of Hg that accumulates in placenta, an average of 60% of the Hg in placenta was in the form of MeHg. The formation of thiol-MeHg bonds is likely responsible also for the placental accumulation because MeHg, like Hg²⁺, is known to bind to thiol-containing molecules—for example, proteins, cysteine, and glutathione (3). Whether this accumulation of MeHg in placenta causes any adverse effects in humans is not known. Studies on mice have shown effects on the activity of selenoenzymes, such as inhibition of glutathione peroxidase, although the placental concentration of selenium was not affected (33). Glutathione peroxidase is an antioxidative enzyme, and high levels are probably needed in the placenta to protect the fetus from the oxidative stress produced during the metabolism of MeHg (34).

We found no correlation between selenium and MeHg or I-Hg in placenta. The selenium uptake in placenta seems to give concentrations within a narrow range, 150–250 µg/kg (24). This range is far higher than the concentrations of Hg (about 1.8 µg/kg as MeHg and 1.3 µg/kg as I-Hg). Interestingly, we found a strong association, on a molar basis, between serum selenium and blood MeHg, but not I-Hg, only in the few women who reported intake of freshwater

fish, and who probably consumed more fish in general than the rest of the women. This probably reflects the fact that fish is a major source of both MeHg and selenium. Previous studies also report associations between total Hg and selenium in blood of people eating marine fish (35,36). Although a protective role of selenium against MeHg toxicity have been shown in animal studies and human *in vitro* studies (2,21), no epidemiologic studies have yet been able to show such an effect in humans. Therefore, the possible protective role of selenium remains to be further investigated.

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